1							-				7-1-9	76	
	Mr	DI	Pre	p of	Plasmi	y DNA	from	Trans	formed	DM1			
		9	cus	ture	starked	mon	colo	m	w1 1	عسره	cella	(see p	revous
			pla	rted	@ 3	sml	\rightarrow	then	30	ml		,	
		2)	mi)1 Pre	p of	plas	nid D	NA C	see	Riage	n		
		9	M	ni Pr	en o	mtacol	A strain						
		3)	DW	esn F	spend	ed :	m ~	كىر 30	TE	111		·	
	N	otes	3 3 3	culti	ring.	of D	ti w	2C 2E	ry sl	ow ·	about		
	•=		21	ለな	o get	from	ر	ar w	redia	to t	urbid		
*		נ	y c	entrifi	igation	to	rellet	AUG	dur	ing MIT) prie	ρ	
		L	SOX	not ,	favora	ble:	DINA N	ot rec	aila	ખેંદો	ble s	.	
			AUG	₽40≠ 7_	(or	suffici	ent a	zuanti	ties	of DNA	ma	<u> </u>	
		n c	<i>st</i>	have	been	iso	ated						
											7		
 							·						
	 	+		 	+		 		1				1

				:		: 1	···		1.	·		- 96 >	7-2
	Diges	hon	4	PEGI	P-N	الن	Age	<u>T</u>	8,	Bsp	EI		
•	and	اسما	DFOX	isb u	ol Bs	PEI						1	
					30								
total non-	<u></u>	ารถ	FP-	N	Boul	from	previo	2VC	exper	ime	ut)	wis	
9. 001		i act	$\epsilon \lambda$ ω	Aas	TO	POEN	n ten	10 .	over	mg	Kut (Hul en:	L yn
7-2-96	2)	MID	L'M	S (Pr	ecipital	la bot	cold	tt3	OH.	, re	suspe	nded	
1 10	(L)	VOI	¢υ,	این	ROFT	(4)	2)-	for	~4 V	v.	Q	37° C	
	عا	Con	cer	untlu	DSP	OXSB	(5 m	(1)	was	cu	t w/		
		Go	o FT	(4	()	nd in	cubat	ed (37°	fo	r	hr.	
		<i>₽</i> \$	7 22	FP-N	((0)	+ WI	BODE	1)0	md	- 15 - 5 F	OXSB		
		*	· Proc	ماء محلم	4	50 ul	total	Vn	ums	r V	xn.		
		,	ncled	nge T	y w	oc Hi	eW ~	har	111	<u>.</u> ن	IP.		
	(۲)	vig	esteu	25 LOX	sp w	as th	111	-ACA			1, ,		
		70	<u>r</u> :	area N	www.es	, , , , , , , , , , , , , , , , , , ,							
			-10-		1	1.5%	00.00	4206	00	Ci	00 V	wl	
	5)			run	bu.	1.3/0	wa	445	2		70 4 7	- 501	
		~	mark	ALL _			1						
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							1						9
			al land		A COLOR	(COOK)					CLR	r in	
		S			<i>,</i>						aga	NORC	
									(
											1		
	1		-										-
													+
									/				-
		1				MARTINES CA FILER (UNTIT)							-
			PFox	58	PEGFP	<u> </u>					!	:	_
	cut	w :	BSPEI		+ Age							!	

		,								7-2	-96	
		6) 0	FOXSB	(Bep	=1 au	t) bay	nd a	md ?	PEGFP	-N -	750 pc	
		·	bond	was	cut	out fr	on of	el an	nd pu	mijied		
			usina	Qia	gen G	el Ext	raction	kit				<u> </u>
			* 7 F	B3 XC	band	= 2	15 mg			2		
			* PE	GFP-N	3 750	pp po	md =	206	mg			
		JJ (F	Do Al	Ented	from	spin	colu	mn,	عمد			
		178	enster	rdid	ni	50)u	LD	3T				
	Liga	tion	of	PFOX	B	and	PEE	FP-1	7	20 pb	fragm	<u>verit</u>
	(EG	FP +	NLS	<u> </u>								
										1	-	
		DThe	follow	rng s	Rigation	on r	eactio	us o	ocre	setup	7 %	
								•			-	
		Tube:	#	1	content				-		CCD.	
	·	1	-		1 jul	cut	PFO)	V V.	- 5 M	Pi	GFP-N	0
	-	 		<u> </u>	trag h.m	ment	2 1	<u>بلا لایر</u> د د	ndaze	1 D.	l lìgas Loe	
		 	+		LL ~	ud lo	47	aa	120	Lange	war.	
				 	٠ ٢٠٠	1000000000000000000000000000000000000	,,0			 		
		2	+	 	101.1	cut 9	Foxse	3+	101	ERFP-	N fragi	ment
			+			ul e		+ 1	5,.0	buffer	7	
		<u> </u>			+ 3	1.5 u	l dd	H, 0				
						vol=						
(^-	Clorton	3				cut	_	SB +	9 ml	ad Ifz	0	
	WY!				L.	backg		~				
					tot.	vol =	10 ml					
· · · · · · · · · · · · · · · · · · ·		2) -	the ak) eve	Conten	क क	ere n	mxed	(excep	t for	eigas	<u>()</u>
		ز	in Ene	èc	entrifuc	jed b	riefly	of the	n ligo	se wo	s add	id_
			7									

	•		. (.)						- ·	-00	
Liqu	ation	(00	nt a)		•			٠ ، ،	7-2	-46 	
·	271	inatio	N r	on	incul	bated	over	night			
	@	14-	15°C								
									7-3	-96	
7	lasmid	HIDI	Pren								
-					led a	n Dr	11 tr	unctorn	nd		
* pellet bacteria	1) 110	100	Canan	C C	Carry	Cu 0 +	7.4.0	(prepo	ned 6	n.	
bacieria	00	1 /	611	1000	1041		2000	10 101	caibe	1	
from cultur	g Ma	ike)	JOHOU	1114) MAI	r pro	+00-0	ومدي	7-1.	96)	
by spinning	- m	Qiac	en c	plasm	101 111	ψι φr	010000	. رين <i>د</i> د ۱۵۰	10°C +	\\\\\\\\\\\\\\\\\\\\\\\\\\\\\\\\\\\\\\	
@ 40 for 10	+	DM.	Cu Cu	iture	yrelde	d mo	re a	XXX 7	ms 1	ivu	
speed 4	1	(~	3X)			1.0			, ,,		-
	2) for	MID'	back	. ,	<u>P1</u>	outer	and	12	buffe	12	
	wer	e add	led '	before	resu	spensi	PN &	not the	Xive	of ce	lls
	3) AS	ter	5 m	mati	ψn =	in ice	the	add	tion	 0 	-
	کح	Hud	ier o	md	subse	quent	inc	whatic	o ne	n ice	<u></u>
	For	151	1	w w	xture	was	s al	iquote	d in	70	
	1 6	اک نماد	i w	1	ml:	m e	ach -	ube 1	or a	total	
		14	tulk								
	4) 71	Jul	*2 (1	ru th	un c	entrifi	raect	@ h	Spe	ed	
			10°	DHH	14/h	mm	crocent	n fuce	(G)	RT Crow	n tem
		S V		100		egailik	het o	WING	ml		
		1.0			MM ⁻	grunn	Juca			6 ° 6	
	-	(DRI	puffer	2				moied	1 43 6	dumn	T
	167.8	Sipern	atant	from	each	tube	var o	47~~~	100	WIWIII.	+-
	 7) ("Jumn	wash	$\frac{\sqrt{1}}{2}$	X 10 W	n Q(Duff	er .	· r. /	· - 17	+
	(8)	DNA	elute	d fro	w co	lumn	<u>Ψ1 Q</u>	t but	ter ((5m1)	+
	<u> </u>	und C	ollecte	lim.	Fool C	1 Cal	iounted	to 50	ש לעולא	zer tuk	41
<u> </u>	9)	AUG	was	ppt.	by	adding	320	Jel 16	ideado:	mol	-
	to	each	tabe		cen	trifug	ing 6	j Si	speed	(RT	}_
	for		a.l		'		1		<u> </u>		1
	 - 30										

Plas	mid MIDI Prep (cont'd)	7-3-96
	10) Ethanol (cold \$7090) was used to wash	DUA
	(sopul \$ / tube) and radiscolved in 75 ul	2 TE 1X
	* 75 ULTE was added to tube I and	
	this same TE was used to resuspend	ppt. DNA
	in the rest of the 9 tures: ultimately	k,
	200 This (priging 10m) separated in 10	tubes)
	is resuspended in 1 tube labelled "PEGFP-N (MIDI) 7-3-96"	l
	" OFGEP- N (MIDI) 7-3-96"	
	11) on aliquot of this prep was then r	un on
100 V	om agarose (1%) gel:	
1001	on agorosa di no	
	Drosing allower association of the control of the c	
	2 2 2 3	
		·
	2.8	
	\$	
	ic)	
	* 10 ul 2 marker * PEGFP-N1 & PGK-neo = 1 ul plasmid	
	* DEGFP-N1 & DGK-neo = 1 ul plasmid	
,		

7-3-96

•											<u>_</u>	22-11	<i>v</i>
	Transf	oma	tio	n of	Compe	tent c	ella u) PF1	OXEGF"	P-N1	lig ati	m	·
* baterio		D C2	علا	+ than	ved_	on ic	e (100	ul al	la/tu	be),	Hun		
used in		a	de	dal	l of	Digation	א לאן	is. to	tube	206	cells		
H8101		2) 0		fourth	trom	form	notice	∞	sou	serfor	Med		
		U	LSIN	V Q+	BS D	Not	to tr	ansfor	m co	els	1 _{ue}	BSDN	ot)
·		ا	\mathcal{L}^{\prime}	Mai	Ko								·
		3)	in	cubat	u on	ice	for 3	o';	warm	0.9	ml L	B @	
			17	70						1			
		4)-	h	ot ev	ock (@ 42	for	145	sec	and	iaf	or 2	1
·		+	he	m a	id e	igatio	n rxr	s. to	extra	(all	iu fi	l nn.	tube)
		5).	ہند	reuba	te @	370	for	1 hr.	; bric	thy saiv	down ? remo	alls 6	SKC.)
		6)	d	ate	<u>σγ</u>	amp	plate	s as	foll	ows:	remo	and al	es in
			Ì	a Co	late	ooul	<u>a</u>	Jul i	nsert	10 ul	insert '	media	10000
		·	ļ	and	contr	ρ\							
				6) 6	treak	plate	W S	00P	5 ul	BS	4 Not		ļ
		٠		A	Jul	BS DI	Vot -	ىر10 <	l tot	al vol	. : W]	ddfL	D ;
				- 14	en o	dded	40 10	Oul	compet	ent c	ells		
	14.3	3)	ىد	autra	te, a	370	Par -			₽ 0	nemig	wt	
		8)	P	cked	24	coloni	es fro	m (plate	<u>wl 1</u>	l LB	insert	-
	8.5	c	w.	d gre	my ?	m d	eture	tubes	<u> w </u>	3 ml	L LB		
			OV	ernig	ert in	shak	ec.						1
												:	<u> </u>
						ļ							ļ
			٠.									<u> </u>	
										ļ	<u> </u>		
													
									ļ				<u> </u>

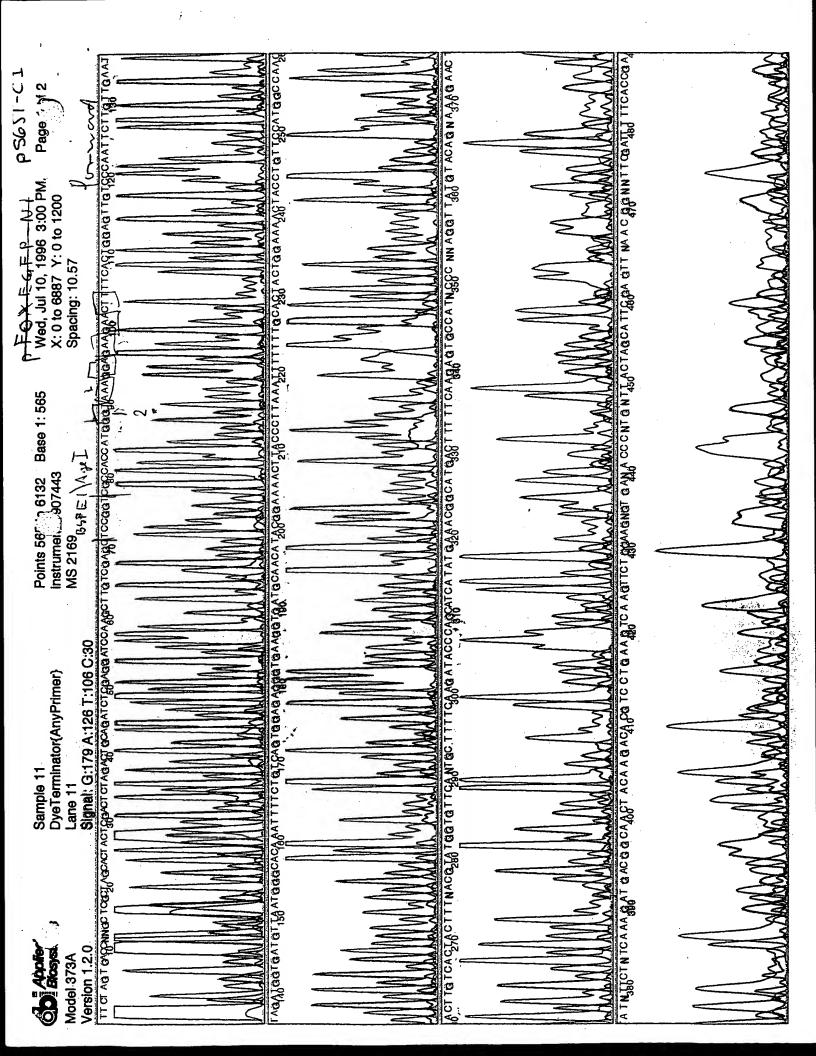
•				•						7-5-	96	
	Minipre	'n of	o Fo	X EGF	2N1	and b	Endonu	clease	Digest	า้อา	·	
•		'										
		Day	- 1 w	11 0	liqu	id cue	ture (from	total	of 24	cultu	es)
		in	Eppi's	and	Spi	n 50	sec.					
		2) 20	ur of	f Sup	ernata	nt bu	Lickin	- 1-20	envert	ing to	We.	
		one	e , +1	ùs le	aves	لىر 50~	l in	tube	; resu	spend	<u>+</u>	
		<u>pel</u>	let i	n rem	airing	med	la by	y vor	texing.		<u> </u>	
		3) 0	ad 3	300ml	何,	US (c	ple ly	sing)	rand	<u> </u> ••		
			ير ٥٥	R 31	H N	1aOAc	17H	5.21	cent	Trugec		
			la so	eed &	r 3					ļ.,		-
		4) L	Jupern	trata	tra	nsferre	10 to	J EL	H . A	niv h	1	
			adder	11 Y	1.100	100%	m com	trifus	6 60	ni soon	d for	3′
		E) -	SCOUNT	to d	CI	permai	tant	ano	unc	h as	1	
		colo	70	% Ft	φH:	really	ton w	ent +	wes	and		
		CON	trifuse	[@ l	ni spe	ed fo	r 5	(do 1	not mix	or inv	ert tuk	*5 °)
		6)	aspir	rate	611	super	matar	ut a	md			
			resu	pend	ed	in 30	الرد	1X	TE			
D	gestion			1								1
	EcoRI	(1)	diges	tion	of 2F	OXEGF	P-N1	<u>. wl</u>	Eco 1	<u>u </u>	rre:	
	ļ	se	tup ,	of 2p	llows:	1				1		15
		-	-	5jul	DNA	(PFOX	EGPN-A	11:00	ater pF	UX SB.	as contr	<u>(10</u>
· .	-	معلي	+		ul RNa	de (10 mg	i wo j			1.	
-	1 W	vaster,	+-{	ر٥.5	ul Eco			<u> </u>	-	-		-
	PY	repared		111		ffer I			-	+		+
	OV	n ia	+ -	3.4		2 volum	\e				+	
· · · .	-	-	<u> </u>	10 11	<u>r 1010</u>	C VUW'	7	-	+			
		-	1	+	 		- · · ·					
		-										
	1		İ	1		1		[

Dia	estion of	PFOX	EGFP-N1	(cont'd		7-5	-96
7				<u> </u>		· · · · · · · · · · · · · · · · · · ·	
•	2) digest	non @	37° was	performed	d for a	thro.	
	3) add	2 ul l	pading d	rje (lex)) to lead	In tube	:
	and	Load a	samples	on 1.	5 90 ag	In tube jarose gu	L
	and	ran @	90 V	•			
		_	11	nauzem ^a			
					VI		
	:	10					
		The state of the s					
		_1					
		======================================		BUNTTLE	(0)	-	
		MC1245	67 89 1011 12 131		#324M3		_
			4 -> 5	24			
	*	2 and 13	= no D	NA			
	1		wrong w	i			
	*	DINA does	not apr	sear diges	sted well	; digest	> 2hr ?
	*	not well	digested	ble used	Buffer	1 7	
			PFCKSB				
:							
			·		i :		
:				·	· · · · · · · · · · · · · · · · · · ·		
							:

	 20: 100 €
Redigestion of PFCXEGFP-NI MUNIPREP	7-8-96
in digestion runs were setup as previously descr	j bed
except used Ecc RT buffer (1x in sample).	. and
only wo. sul proxsis as control (r	an out
of proxsb)	
2) digested @ 37° for ~2 Ans. 20 min	
and located onto 1.5% sel @ 100 V :	
014 2mpx (1 31/2 1 3 /0 det (3 100 V c	
	* 175 band
	not seen;
	low molar
	concentration?
######################################	
MCI 22 EEBYH 22 EEBYH	
* loaded 20 ul 1x marker	
* control = ~0.5 ul pFOXSB cut w/ EcoRI (1	2 ml)
* all spl loaded were ≥ 12 ul	,
* lanes between 22 à 23 were empty	
* presence of 050 bp band in 24 but	no 175 ba
band is observed for all spl; this many V	now remitted
from incomplete digestion and dirty prep	P
from incomplem orderings and anish bish	n d
will make a new prep of colony 124 a	
<u>u digest</u>	

•		•				-						
•					····				·	7-8	- 96	
	Rec	ueture	of,	colomy	24	for m	impre)				
•										•		
) ~	+ 1	revile a	doman i	_B_(3 ml) in	to 2	cultu	ne	
		10 - 101) - A	0.000	() 5	3 + av	lan					
		- n()	- Cach	os. Pot	ing lo	תקיי	and I	and	dia int	*	
		2) 1	Carra	du sala	Total	1119			+ 1	only	<u> </u>	
		<i>t</i> t	be a	of cold	my 2	an	Lu A	P J	40 7	arvi		
		tu	ve w	1 40	10	recul	ruse 1	rep	as a	220		
	···	Δ	econd	time	amd	m	wall	Tu	vis (e	31		
		0	vem	ight	unte	turi	rid;					
			addition	nal tu	bes (for Ma	ike):					
		!		5 Not	1							
			(b) ~(e	,K-nec								
			# for	these	inoc	ulated	a co	long.	from	each		
			plat	te an	d cul	tund	over	night	in	3 ml	LB	tar
	Ч	iniore	o(Qio	gein)	f coloi	ny 24 9	BON	tÈ	7.	9-96		
		GK-W	30	3- 7-0		0						
* back	evia =	0 20	llet h	ncterio	bu s	pinnin	Q (50	eod L	1) @	4° C	for	
si Buza		V	1	i co cod	3	evnata	nt mt					
11810			, a	2-2-0	211.0	n PI	on	a	لعومييوء	nd .	sellet	
		② a	aa	300 JUNE	Emil		- CVII		Marra P	7		1
		7170	unster	to	CHOI	5	٠٠١٠٠		2 À	inalle	+1	1.
		(3) a	30 30	عدود	Buto	P2	gent in	· mx	ana.	Music	<u> </u>	
		(9)	KT_	for s	C .		C-			- 1		ļ
				ni 11ed	-	i	1		myen	2-6	X	-
		omo	inc	etolle	90	ice &	br 10		1		1	
		(3)	worker	ged (a sui	spee	d for	15	j su	pernato	mt_	-
·		Mo.	red to	D 20	tip	colum	n (e	quilib	ratecl)			-
	(Dwa	sh L	LXL	ml B	ruffer	QC	ther	DN	A elut	<u>ad</u>	-
		u	1 800	کیر (Buffer	ÜF				<u> </u>	<u> </u>	
		<u> </u>			.))							
	i	1	1	1	1	1	•	1	•	•	•	•

	Mir	<u>ù prep</u>	(oon)	t'd)			,			7-	9 - 96	
-		3]	AUC	ppt.	wl	560	e i	popro	pamal	(0.	7 vol)
		and	pute	yuged	(a) -	en spi	zed f	<u>თ 30</u>	,			
		(8) D	NA W	ashed	wl	1 ml	70	olo i	told	Etoh	•	
		cent	trifuge	15 50	ec. 0	ind /	edisso	lued	in	20 w	1 X	TE
			<u> </u>									
	+ Di	gestic	o C	of Colo	ony a	24						
		1) D	igestic	n se	tup	as fo	llows	(2	2mx	- mad	٤)	
			11	of Du	AU	(pfox	EGFP	-N1)				
						Buffer	<u>ل</u>			·		
				2 E								· · · · · · · · · · · · · · · · · · ·
				e d	1							
				el tot								
			igestec) for	1 hr	. @	37	C				
		2) 10	aded o	DULD	1.5	o aga	rose	gel:				
				سا			11					
		· · · · · · · · · · · · · · · · · · ·	* 12	ul c	digestro	n spl						
		<u> </u>	1 4						· ·	,		
											47	
											* Dry	
					· (1)						. 646	
						v			2.		bod	
_			e con								1119	rime
		•			4			野狗			for	10.0
U C												n for
					1						5	
		-				17	ā			4		
	TURE: 20/30 SE: . 2. 07-09-1996	-CK: 1 UHI T	157 Serme 2.9 1 Ft 3-01070 F	O ILE: NTILED)		FPOSURE: 1.1	SEC. EL-1:10 96 TEL D		901075 71LE: (U	(<u>7 - 20)</u>		
	# : Jaovo		GO! 02:		i	1 gar	1000	М	12			1; 1
	* Junk	n !	S P	1.XX (7) 6		* 0	11 rudi	gest			le reign	don
	. 5 1.	IN (IC)	nl.	:		٠. ٨.	ila	×- +	Mi All	Orimi D	רודוויייים	+w.



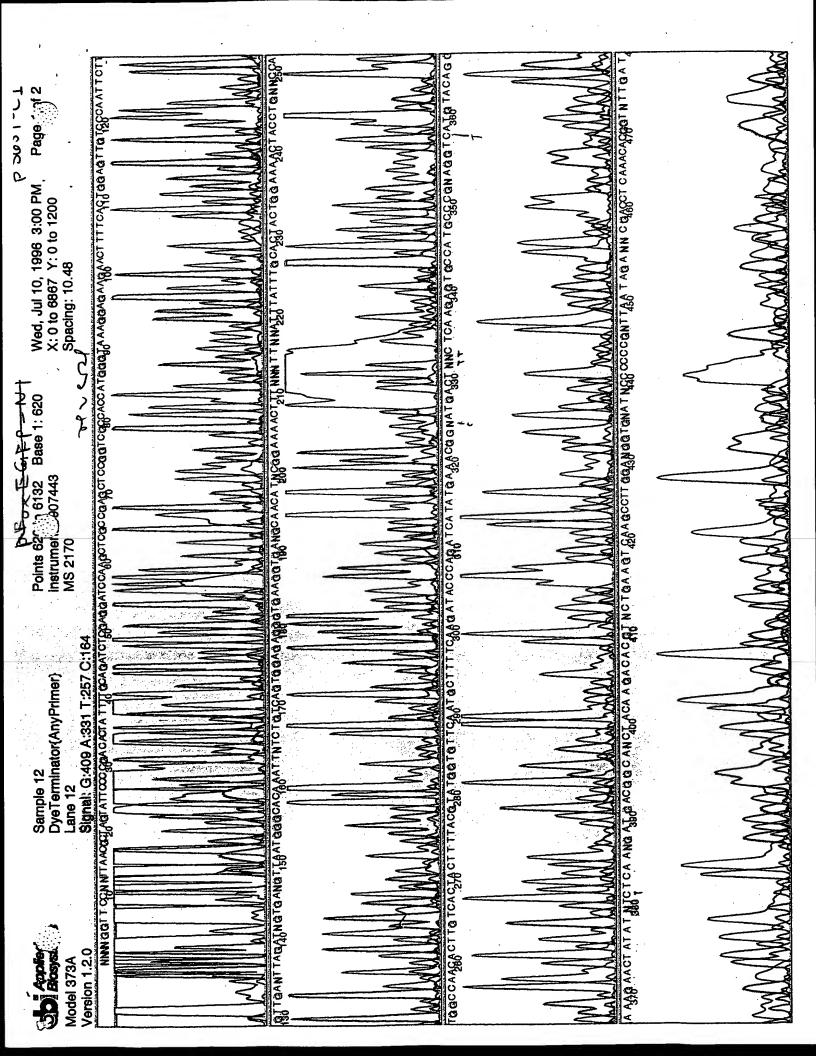


Sample 11
DyeTerminator(AhyPrimer)

Points 56 0 6132 Base 1: 565 instrume 907443 MS 2169

Wed, Jul 10, 1996 3:00 PM, X: 0 to 6887 Y: 0 to 1200 Spacing: 10.57

Page 3 of 2





Model 373A Lane 12 Signal: G:409 A:331 T:257 C:164
Version 1.2.0 Signal: G:409 A:331 T:257 C:164

EJINNIAC GINA MAY T GGA A ACCAAN C T NINGGA AA OT GGAN CT CAN CGLOT AACTCCA HIN C AANCO NO DyeTerminator(AnyPrimer) Sample 12

Points 620 6132 Base 1: 620 instrumet. 907443

Wed, Jul 10, 1996 3:00 PM. X: 0 to 6867 Y: 0 to 1200 Spacing: 10.48

Page 7 2

- Sac I Digest p FOXEGFP-N1	7-9-96
1) the following digest was setup:	
10 MA (PFOXEGFP-N1)	
1 ul BSA (final conc. 2x)	
4 ul Sac I	
5 pl Buffer I	
30 ul adth 0	
Soul total volume	
y a second digestion we sha RI was	setup as
neviously described (1 ul Edo RI	: 1 ul DUA); both
digestions performed in PCR mach	ine @
37° for 4 hr.	
	7-10-96
Eco RI digest of PFOXEGFPNI was ru	m on 1.5%
agarose gel:	
	noutres à 12 ul non. Spl
* colony * bod dig	estion can see a
24 #1 lot of un	digested plasmid
	ewe the partial digest
	> 1000 bp
- this	may have resulted
From	incomplete digest to
make	850 + 175 => 1025b
5 FOSURE: 11 21 SEC. Bu-1:1 UHL TE: 113 GAMM-1:1.50	(GFP ENLS) band
COSENSE	

•

•	C.	P_	of.	p FO	CEGFP-	N1					7	-10-96	2
		0	Ċ	IP C	M. 4	4 5	ac I	digest	ed	vector	was		
·		S	S	etuo	as fol	(ows:	(add	ed di	rectly	to di	estion	nxn.)	
				30	لىرد	10 X	CIP	Buffe	٧				
		İ			الر	dd H	.0						
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			1		el	total	vol.	الأع	cubate	@37	for	30'	
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				2 ul	insert	- (B-a	lobin is	ntron)				
				1 111	ligas	(p-g	175 pp	C4000	w 000	ml)		
			*	1 11	lian	se buff	er C	10x)				
				5 ul	dalt	O						
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1		,		• •		-: 13. 1						44

(1.1.1. •)		
·M	limprep of groxsB	7-11-96
	i) as described in shera's protocol, except	<u> </u>
	i) as described in shera's protocol, except put on ice for \$5' after adding Na OAC ((3H pHS,2)
	2) after adding 100% EtOH, put in methanol	/Cb,
·	2) after adding 100% EtOH, put in methanol both for 15'; centrifuge @ Di speed for 3) TNA resuspended in 30 ul 1x TE	37
	3) THA resuspended in 30 Il IX TE	
	No I Digestion of primary plasmids used to a	onstruct GFP
	plasmid	
A COALÍSMU	* sequence data of proxe6FP-N1 showed that	the
data Parino	wrong placemed many shave been used to	construct
-n west page	GFP plasmid; cutting w/ Nco I should	determine
er war page	I hadden the about thousand (-thether) o	Ma.
	not the wrong old plasmid (p865T - used to construct plasmid. The wrong plasmid.	C1) was
<u> </u>	and to construct of plasmid. The wrong of	asmid
	- SIST- C1 wield an extra law w	eight
	ps657-C1 will wield an extra low w fragment (167 bp).	
	1) Digestion van. setup as follows:	
	1) Sigestion VXV. Sexual as forious?	
	5 ul DNA * for commercial plasmi	do
		5T-C1
	I I'V NOT only I I'V DIA was	1 . 1
<u>nux</u> made	1 re Buffer 4 and added to 4 rel do	
11100	2.9 ul datto and this was added to	5.11
	10 ul tot. vol. of mix we a tot. r	rn. vd. d
	10 pl	71.1
		18 (positives)
···		Chai nota)
	@ 37° for 1 1/2 hr.; then stored in	har is a
	Greezer	(MD) cree)
	Greezer + digertion of PEGFP-C1 (prep); PEGFP-N and DEGFP-N (manethylated) was perform	red
	and DEGEL- in Cardinating I man baild.	

Not I NCOI Digestion (cont'd) i) digestion runs, were run on 290 agarose gel: (1201) Dame order : Marker 2) DEGFP-C1 (commercial) 3) pSGT-C1 (com) 4) pEGFP-C1 (prep) 5) pEGFP-N (methylated) 6) PEGFP-N (MIDI prep, unmethylated * used 20ml marker * ~ 12 ul sol laded > presence of extra 167 by fragment indicates minis > start over of DEGFP-C1

7-12-96

Annualing of Oligos Hosses! I) added I see (I set) of oligo Mg528 and M6529 (M6529 into rixn. W 2.5 set Buffer H conc. of (Coehningen Mannhuim) to a total rixn. vol. 1 yel of 25 set Doiled in water both for ~1 minute then lever in water both as it ago tempe. equilibrated to RT + no kinace 3) pt (O 4°C for & Inr. before using Digestion of pe6FP-C1 (prep) Differ of Deffer-C1 (prep) 2.5 set Bsp E1 2.5 set Bsp E1 2.5 set Buffer 3 20 set of total volume 3) incubate (23° for 4 Mr. 3) phonol / chloroform and ethanol pt. the DNA reswapend in 30 set 1xTE			
Annualing of Oligos # H6528: 1) added 1 ug (1 ul) of oligo MG528 and M6529 @ MG529 into rxn. w/ 2.5 ul Buffer H conc. of (Boehninger Mannheim) to a total rxn. vol. 1 ug/ul of 25 ul 2) boiled in water both for ~1 minute then kept in water both as it ago tempo. equilibrated to RT # no kinase 3) put @ 4°C for a In. before wing Digestion of pE6FP-C1 (prep) 1) digestion rxn. setup as follows: 10 ul pE6FP-C1 (prep) 2.5 ul Bsp E1 2.5 ul Bgl II 5 ul Buffer 3 3 oul add 10 lume 2) in autote @ 37° for 4 M. 3) phanol/abbooform and ethanol pt. the DNA			•
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# M6528: i) added 1 ug (1 ul) of oligo M6528 and M6529 @ M6529 into rxn. w/ 2.5 ul Buffer H conc. of (Boekninger-Mannheim) to a total rxn. vol. 1 ug/ ul 25 ul 20 boiled in water both for ~1 minute then beept in water both as it ago temps. equilibrated to RT # no kinase 3) put @ 4°C for & In. before using Digerion of pE6FP-C1 (prep) 1) digerion rxn. setup as follows: 10 ul pE6FP-C1 (prep) 2.5 ul BSP E1 2.5 ul BSP E1 2.5 ul Bgl II 5 ul ddH20 50 ul total volume 2) in avvote @ 37° for 4 M. 3) prenol/chloroform and ethanol pt. the DNA	 		-12-76
# M6528: i) added 1 ug (1 ul) of oligo M6528 and M6529 @ M6529 into rxn. w/ 2.5 ul Buffer H conc. of (Boekninger-Mannheim) to a total rxn. vol. 1 ug/ ul 25 ul 20 boiled in water both for ~1 minute then beept in water both as it ago temps. equilibrated to RT # no kinase 3) put @ 4°C for & In. before using Digerion of pE6FP-C1 (prep) 1) digerion rxn. setup as follows: 10 ul pE6FP-C1 (prep) 2.5 ul BSP E1 2.5 ul BSP E1 2.5 ul Bgl II 5 ul ddH20 50 ul total volume 2) in avvote @ 37° for 4 M. 3) prenol/chloroform and ethanol pt. the DNA		Annealing of Oligos	
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3) menol/chloroform and ethanol ppt. the DNA		50 el total volume	
3) menol/chloroform and ethanol ppt. the DNA		2) in abote @ 37° for 4M.	
resuspend in 30 ul 1XTE		3) when I charoform and ethanol for the DNA	
		annound in 30 ul 1xTE	
		The state of the s	

Ligation of PEGFP-C1 (cut) w/ oligos 7-12-96 MG528/MG529 # 3 dilutions of oligos were made: 1:10 1:100 1:1000 1) * ligation rxn. set up as follows:

1 ul cut plasmid (PEGFP- C1 aut w/ BSPE1; Bg/II)

1 ul oligo dil'n (1:10; 1:100; 1:1000)

1 ul TH DNA ligase (400000 ul/ml)

1 ul ligase buffer (10x)

6 ul dolto

10 ul tot. vol.

* control : 1 ul cut plasmid + 9 ul dolto > 10 ul tot. vol. 2) Incubated @ 4-150 overnight Transformation of DMI allow/ 7-13-96 PEGFF-C1 ligation rxn. 1) DHI allo thaved on ice (>100 ul allo) and ligation rans. added to cells; in autate in ice for 30' then heat shock cells

2) in cubate @ 37° for 1 hs; & plated on

LB + kan plates which were incubated @ 37° overnight * 100 ul were plated on each plate of very low transformation (1-2 colonies) in 1:100 è 1:1000 diln.; no colonies in 1:10 dil'n); will do a new ligation but use HBIOI cells instead.

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Ligation	PEGF	P- C'	1 (cu	t) ω	1 olig	202		7.15	.96	
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· <u> </u>	tin(II di	gistion	06	DE6FP-	m 2% agarose gel @						7-17-96				
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Tho I fille I digestion of PEGFP-CI ligation 7-18-96

1) digestion non. run on 1.590 agarose gel @ 100 V: 4 20 ul marker; 12.5 ul spl. loaded; overloaded control (contains 5 \ rel pEGFP-C1 prep; next time only use I il athered to 5 ml) * col. 2 =4 & appear slightly larger than control but cannot say definitively since resolution of 50 bp in crease in correct construct is hard to resolve # will passage 2 4 through DM1 and when
these colonies are cut w/ Age I & BSP EI will also cut parent vector wi same enzymes for a control; again, correct fragment should be ~50 bp larger than control fragment

· Tra	instrumation of DM1 w Suspected pEGFP-CI ligation 7-18-96
700	sitives
+	1) transform DU1 alls wi DNA from whomes 2 and 4;
	heat shock @ 42° C for 45 sec. in cultate in LB
	@ 37° for 1 lm. and plated on kan plates (100 ul cells)
	pEGFP-C1 ligation & digestion 8-5-96
	* Cultured colonies 294 in Kan + LB 3ml
	* ministep of culture 4 and digestion w Age I
	* PEGF?- CI ligation run was digested w/ Age I as
	Collais:
_	DNA Buffer I Aget Robert A ddH20
	DEGFR-C1 Dig 40 ml 10 ml 4 ml 1 ml 45 ml
	2) JEOFF-CI (gont) Tue Till Till Till
	* digested overnight @ RT
	8-6-96
	* EtOH ppt. Age I digest in ran. is digested will BSP EI
	for 4 hr. @ 37°
	* minipreps done on colory 2 fellowed by Age I digestion
	as described above
	> 2 control ring setup as above
	y for Bop ET diopsition, lighting our diousted and as follows:
	@ 374 for 4 hr.
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* started liquid culture of pE6FP-N1 in 3 ml LB+amp + numbered I'me pEGFT-N and digested as Gollows: 5,18 DNA (DEGFP-N) 3 ul O. Jul RNoor A 30x 30 jul Jul EcoRI 30 ml In EcoRI Buffer 87ul 2.9 ul dath, 0 roul tot. vol. * incubote @ 370 for 4 hr * DNA used was resuspended in 30 ul 1x TE # for control, used I ul proxsB DNA (2 controls made) * for culture used to makes. TNA for this digestion, note that tures 1 2817 were clear (i.e. apportently, inc bacteria) and so should be regatives on gel : # digestion run on old. Staganose gel: * 7 definite positives The has 850 & 175 band # 5,79-11, 20 8 22. possible positives include #45/3 13 all the E-POSURE: 1.: FE1. BLACKS 0 ATTE: 128 Same(1).85

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Ligation of proxsB & PEGFP-N 750 fragment 8-8-96 Label Contents lig. BODEL OUT & CIP ddt.0 PEGFP-N Smag ligase Iul Zul 200 5 ul Sul £1.5ul 10ul 10 ul 1.58 (control) C 5 hr. * rxn. run @ RT for * transformed into HB101 cells i plated amp Etatl ppt & digestion w/ Bsp EI of colony 4 (from underday) will amalyze if positive (has 750 band) on yesterday) will analyze if 1.590 agarose * DUA lost during pot #E1 6.31 SEC. BLACK: 0 WHITE: 91 6-MYNG 1.90 18-06-1895 TIPE: 19:14 IDN 569-11460 FILE: (UNTILED)

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* PEGFP-N1 from minipres digested w/ Sac I: 27 ul DNA (#10 DE6FP-N1) 1 nd BSA 1 ul RNam A 4 ul Sac I Jul Buffer I 12 ul adt 20 50 ul * digested @ 37° for 4 hr. * reculture #5 é # 10 into pour fresh LB+ amp (3 me) * PEGFPNI-SacI digest CIP XX @ 37° for 30' WI 1 M CIP
In Sollowing ran: 50 M SacI digest 30 ul 10x CIP buffer 1 ul CIP 219, le datte 0 300, le 6+. vol. * CIP TXN Stop by adding: 39 ul dd H, O 40 ul 10XSTE and heating @ 68° for 15' 20 of 10% \$DS 400 ul tot. vol. W Sac I digest * extract as phenol/chloroform ax; Eto+ ppt & resuppend in 15 le TE (1X) * run on 190 agarese gel & purified for ligation revis to assay concentration (Int DNA assayed) 6 pl monker (1x)

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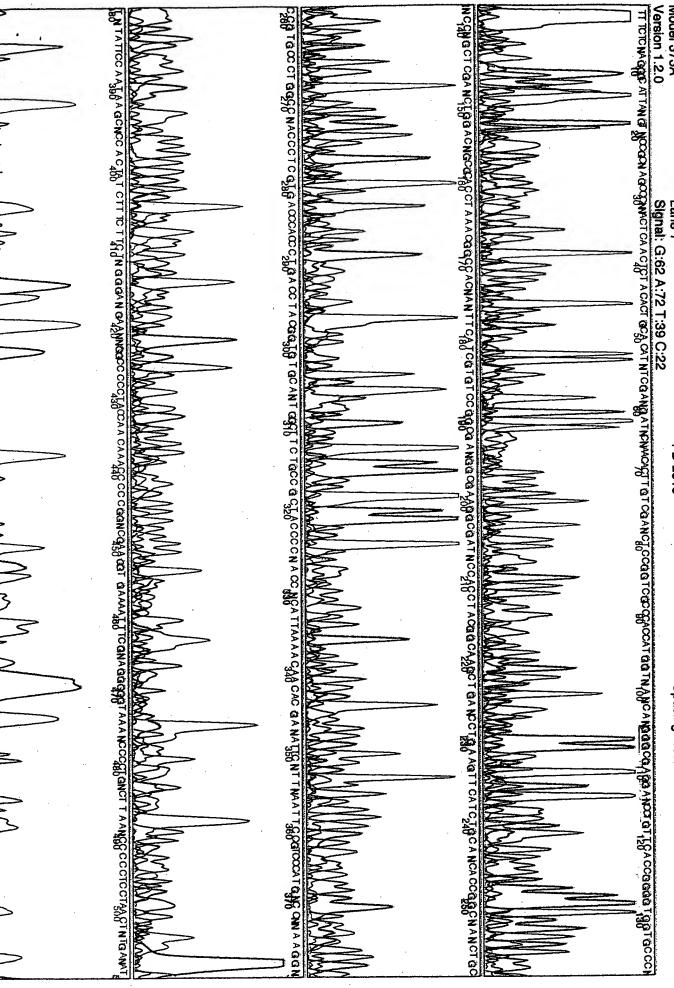
Model 373A Sample 07.1

DyeTerminator(AnyPrimer)

Points 60(-) 6184 Base 1: 604 Instrument #907443

Wed, Aug 21, 1996 2:59 PM X: 0 to 6858 Y: 0 to 1200

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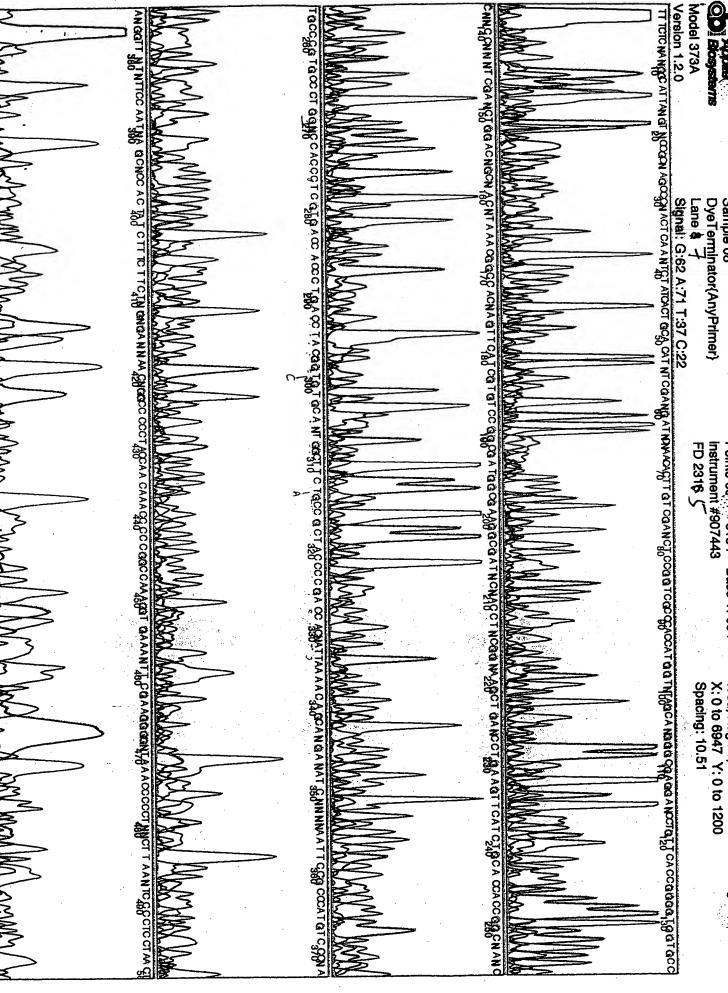


Points 60 6184 Base 1: 604 instrument #907443 FD 2315

Wed, Aug 21, 1996 2:59 PM X: 0 to 6858 Y: 0 to 1200 Spacing: 10.65

Points 60 6184 Base 1: 604 Instrument #907443

Wed, Aug 21, 1996 2:59 PM Page of 2 X: 0 to 6947 Y: 0 to 1200 Spacing: 10.51





Points 6((-)) 6184 Base 1: 604 Instrument #907443 FD 2316

Wed, Aug 21, 1996 2:59 PM Page of 2 X: 0 to 6947 Y: 0 to 1200 Spacing: 10.51

Sample 08

DyeTerminator{AnyPrimer}

Model 373A

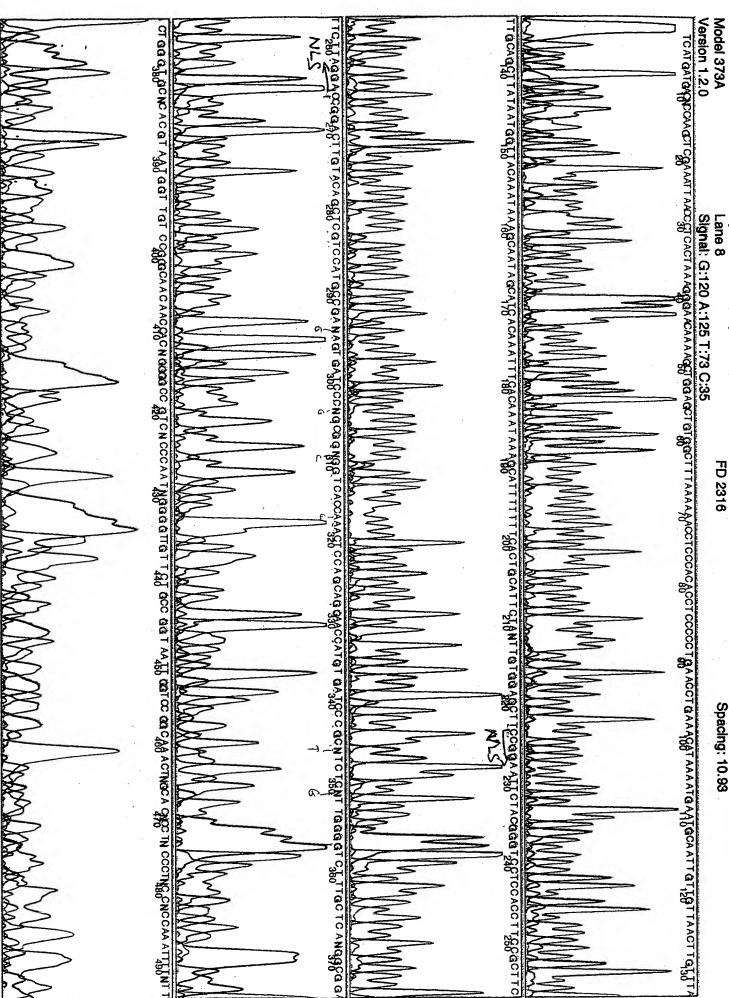
Version 1.2 0

Signal: G:62 A:71 T:37 C:22

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Sample 08.1

DyeTerminator(AnyPrimer)





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DyeTerminator(AnyPrimer)

Model 373A

Lane 8

Version 1.2.0

GC @3465 CTTTWAAA515 CCCCCTNAS GCNCC 155CC CTTTQTAGGN

Points 69 6184 Base 1: 692 Instrument 907443 FD 2316

Wed, Aug 21, 1996 2:59 PM Page 7/ 2 X; 0 to 6561 Y: 0 to 1200 Spacing: 10.93

Obj Applie | DyeTerminator{AnyPrimer} | Instrument #907443 | X: 0 to 6575 Y: 0 to 1200 | Model 373A | Lane 令 8 | Yersion 1.2.0 | Signal: G:118 A:125 T:73 C:34 | Yersion 1.2.0 | Signal: G:118 A:125 T:73 C:34 | Yersion 1.2.0 | Signal: G:118 A:125 T:73 C:34 | Yersion 1.2.0 | Signal: G:118 A:125 T:73 C:34 | Yersion 1.2.0 | Signal: G:118 A:125 T:73 C:34 | Yersion 1.2.0 | Yersion 1.2.0 | Yersion 1.2.0 | Yersion 1.2.0 | Yersion 1.2.0 | Yersion 1.2.0 | Yersion 1.2.0 | Yersion 1.2.0 | Yersion 1.2.0 | Yersion 1.2.0 | Yersion 1.2.0 | Yersion 1.2.0 | Yersion 1.2.0 | Yersion 1.2.0 | Yersion 1.2.0 | Yersion 1.2.0 | Yersion 1.2.0 | Yersion 1.2.0 | Yersion 1.2.0 | Yersion 1.2.0 | Yersion 1.2.0 | Yersion 1.2.0 | Yersion 1.2.0 | Yersion 1.2.0 | Yersion 1.2.0 | Yersion 1.2.0 | Yersion 1.2.0 | Yersion 1.2.0 | Yersion 1.2.0 | Yersion 1.2.0 | Yersion 1.2.0 | Yersion 1.2.0 | Yersion 1.2.0 | Yersion 1.2.0 | Yersion 1.2.0 | Yersion 1.2.0 | Yersion 1.2.0 | Yersion 1.2.0 | Yersion 1.2.0 | Yersion 1.2.0 | Yersion 1.2.0 | Yersion 1.2.0 | Yersion 1.2.0 | Yersion 1.2.0 | Yersion 1.2.0 | Yersion 1.2.0 | Yersion 1.2.0 | Yersion 1.2.0 | Yersion 1.2.0 | Yersion 1.2.0 | Yersion 1.2.0 | Yersion 1.2.0 | Yersion 1.2.0 | Yersion 1.2.0 | Yersion 1.2.0 | Yersion 1.2.0 | Yersion 1.2.0 | Yersion 1.2.0 | Yersion 1.2.0 | Yersion 1.2.0 | Yersion 1.2.0 | Yersion 1.2.0 | Yersion 1.2.0 | Yersion 1.2.0 | Yersion 1.2.0 | Yersion 1.2.0 | Yersion 1.2.0 | Yersion 1.2.0 | Yersion 1.2.0 | Yersion 1.2.0 | Yersion 1.2.0 | Yersion 1.2.0 | Yersion 1.2.0 | Yersion 1.2.0 | Yersion 1.2.0 | Yersion 1.2.0 | Yersion 1.2.0 | Yersion 1.2.0 | Yersion 1.2.0 | Yersion 1.2.0 | Yersion 1.2.0 | Yersion 1.2.0 | Yersion 1.2.0 | Yersion 1.2.0 | Yersion 1.2.0 | Yersion 1.2.0 | Yersion 1.2.0 | Yersion 1.2.0 | Yersion 1.2.0 | Yersion 1.2.0 | Yersion 1.2.0 | Yersion 1.2.0 | Yersion 1.2.0 | Yersion 1.2.0 | Yersion 1.2.0 | Yersion 1.2.0 | Yersion 1.2.0 | Yersion 1.2.0 | Yersion 1.2.0 | Yersion 1.2.0 | Yersion 1.2.0 | Yersion 1.2.0 | Yersion 1.2.0 | Yersion 1.2.0 | Yersion 1.2.0 | Yersion 1.2.0 | Yersio A CT GG GT GG T AA TABATTTGTTCCARGOCA A CAACARACNICAGG CACCC CAALAGG GG GTG TALAT GCT GGT AA TT CAT CO CARACINANA CTNOWN CONTRACTOR AND THE CONTRA тасақұтатаатааұтасааатааддоаатассаұқасааатт PERSONN OLOGIEL LOLIGIODO PERSONO PERS Points 68 6184 Base 1: 692 Wed, Aug 21, 1996 2:59 PM X: 0 to 6575 Y: 0 to 1200 Spacing: 10.91

Points 69 7 6184 Base 1: 692 Instrument #907443 RC 2317

 Wed, Aug 21, 1996 2:59 PM
 Page of 2

 X: 0 to 6575 Y: 0 to 1200

 Spacing: 10.91

Sample 09
DyeTerminator{AnyPrimer}
Model 373A
Version 1.2.0
Signal: G:118 A:125 T:73 C:34
Tac aggat crtrayas tricccc y Nat acc tggcrt coasy, and tack the coasy, and the co

Model 373A Version 1.2.0 CO Apple возокоот завтата в постоя в 1936 ком в посто в стори в постоя в п APABACCAAT ANAA ACTOGGGCATAG TGGAGACAGANA ANACTCITIGGGGGTTTCTGG TAGGCACTGACTCTCTCTGGCTATTGGT288 ATT ACCET AGENT AGTORAGIOT AGACT GEAGATOTOGAGGATECAAGCILIGTOGAGCTETGAAGTTGGGTGAAGTTGGGTGAGGCAGGTTGGGTATOAAGGGTTAAA Sample 19
DyeTerminator(AnyPrimer)
Lane 19, \(\)
Signal: G:352 A:485 T:305 C:158 Control part PD 232 Instrumen. "907443 TCCCACCTT AGGCTAGCTGGTCTGAGCTCCGGTCGC



Sample 19

DyeTerminator{AnyPrimer}

Points 68 6184 Base 1: 691 Instrument 907443 Control pGEM

Wed, Aug 21, 1996 2:59 PM X: 0 to 6503 Y: 0 to 1200 Spacing: 11:03

Model 373A

Version 1.2.0

Signal: G:352 A:485 T:305 C:158

FT ACCCCASS ACATNA A NSG NC ACAATTSJ TOGA TTOT

Ob Applie васах тасявост ховасух астахсссивах и техто от положно в делохосто в положно в председения положно в положно в LIVISTABLIVICO COLCICIO CO CON CONTRACTO LI DOCO LO NO NO NO CONTRACTO CONTR Version 1.2.0 Model 373A géno son vastelles de var var de la varante de la varante de la la de la constanta de la la constanta de la co TITERSE POUND LANGE TO THE BEYOND BREADER BEIND BREADER BEIND BOND CHARGE TO THE BEYOND TO THE BEADER BOND BEIND B Signal: G:358 A:486 T:319 C:157 Lane 18 Sample 18.1

DyeTerminator(AnyPrimer) Instrumel 907443 FD 2326 Points 6900 6184 Base 1: 690 X: 0 to 6631 Y: 0 to 1200 Wed, Aug 21, 1996 2:59 PM Spacing: 10.82 PtoxE6#7~N& Page of 2

Version 1.2.0 Signal: G:358 A:486 T:319 C:157 Model 373A

Lane 18 Sample 18.1

DyeTerminator(AnyPrimer)

Points 6() 6184 Base 1: 690 Instrument #907443 FD 2326

Page _ of 2

Wed, Aug 21, 1996 2:59 PM X: 0 to 6631 Y: 0 to 1200 Spacing: 10.82

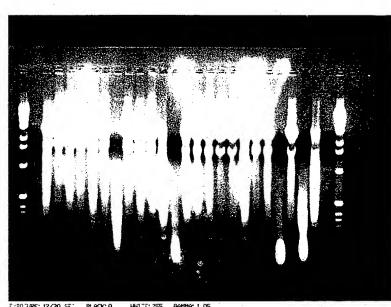
Markes frag. -> used 50 ng vector insert 3 is invent * +182 rge promoter (rat glucagon) * PEGFP-N2 = du dide diget only w1 pos. carrier DNA: digest , men pt. 21 EtOH add In ERNA they continue plasmid prep > dble CsCl (2x) & PESFP-N2

* also reculture & do 20 - tip of PEGFP-NS

* always leave 1 ul of DNA from prep.

* transform 1 ul DNA for a plate for Barn HI / Hind III digest se Buffer B: Inc. exp. level & Tour sens of system whon

. •⊕ + minis 78679-N2 -482 rglc 7- element of Eco RT pier minis for pE(enFPN2-482-21c 2 elment



E:90,7ure: 12/30 SET. 9LACK:0 WHITE: 255 GAMMA: 1.05 147E: 08-25-1996 TIME: 09:56 12# 569-01698 FILE: (UNTITLED)

B ...